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Ottawa Hull K1A 0C9

(21) (A1) 2,182,686
(86) 1995/01/13
(43) 1995/08/10

(51) Int.Cl.[6] A61K 49/00; A61K 49/04; A61K 41/00

(19) (CA) APPLICATION FOR CANADIAN PATENT (12)

(54) Agent for Marking Bodily Tissues

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(30) (DE) P 44 03 789.9 1994/02/03

(57) 10 Claims

Notice: This application is as filed and may therefore contain an incomplete specification.

2182686

Abstract

The invention relates to the use of colored NMR-, x-ray- or optionally dye-containing ultrasonic-imaging substances for marking bodily tissues.

Agent for Visually Marking Bodily Tissues

The invention relates to the object characterized in the claims, i.e., the use of colored NMR or x-ray contrast media or of optionally dye-containing ultrasonic contrast media for the production of a diagnostic agent for visually marking bodily tissues.

With increasing refinement of radiological imaging, increasingly smaller changes are detected which in some cases to a surgeon are not reliably palpable either in clinical examination or during surgery. Thus, structures up to 1 mm^3 can be made visible. Such changes can also be difficult for pathologists to find since, for reasons of time and money, the very expensive millimeter-by-millimeter working-up of major tissues is generally not possible. This is especially important when minor malignant growths are detected in early stages of malignant growths, such as in-situ carcinomas (which almost routinely are not palpable), and for detecting reliable excision in the case of radiologically unclear changes, where there may be no histologic indication of a malignant growth. As examples of changes that must be marked pre-operatively, there can be mentioned: nonpalpable suspect changes that are found during mammographic screening or small subpleural changes that must be excised to rule out or detect metastasis in order to decide on a course of treatment in patients with sarcomas or testicular tumors.

To ensure that such changes are found without unnecessary traumatization or removal of sizeable healthy surrounding tissue and to ensure reliable correlation with the histopathologic findings (including for forensic purposes), such changes must be marked pre-operatively under so-called x-ray, ultrasonic, computer-tomographic, or nuclear-spin-tomographic control.

For radiologically controlled marking of tissues -- after visualization of the lesion with the corresponding radiologic process -- mainly the following techniques have been used to date:

1. Marking of the skin overlying the tissue by an object or by dye.

2. A needle or wire (generally with a way to anchor it) is directed to the focus and left in place until surgery is performed.

3. The tissue to be excised is marked directly by injecting a dye.

The above-mentioned techniques are associated with various drawbacks, however. Thus, method 1 is inaccurate in the case of lesions in subjacent tissue, especially in the case of tissue with considerable respiratory mobility (e.g., breast cancer). Method 2 overcomes this drawback, but this is offset by an increased risk of infection, especially if there is an extended period between diagnosis and surgery. It is uncomfortable for the patient, and in addition, there exists the danger of dislocation despite anchoring, as well as the danger of coming off during surgery and thus leaving a sharp foreign object in the

tissue [A. W. M. C. Owen and E. Nanda Kumar: Migration of Localizing Wires Used in Guided Biopsy of the Breast. Clin. Radiology (1991) 43, 251. J. B. Bristol, Jones P. A.: Transgression of a Localizing Wire into the Pleural Cavity Prior to Mammography. Br. J. of Radiology (1981) 54, 139-140. L. S. Gormly, L. W. Bassett: Pre-biopsy Needle Localizing, Ductography and Pneumocystography. In: Mitchell, G. W.; Bassett, L. W.: (eds): The Female Breast and its Disorders. Baltimore, Williams and Wilkins, 1990].

The most frequently used method at this time is method 3. Generally, in this case, an injection needle is inserted directly into the focus while being monitored by x-ray, and the appropriate dye is injected. In this case, however -- especially in the case of small lesions -- it is not ensured that the dye has actually been injected into the lesion. Thus, it often happens that the die is injected right next to the focus. To locate the retention site of the dye exactly, the latter is therefore generally mixed with a contrast medium (NMR, x-ray), which can be readily detected in a corresponding imaging process, and the exact position of the dye also can be deduced from its spatial position. As dyes for marking tissues, on the one hand, to date water-soluble dyes, such as indigo carmine or methylene blue, etc., or non-soluble substances, such as suspended sterile activated carbon, have been used.

Water-soluble dyes have -- apart from a few allergic reactions -- the advantage of acceptable compatibility. They are readily visible to surgeons and are completely excreted. Their

main drawback is that they diffuse into tissue very quickly. Even in the case of a relatively short interval (even as little as 1-2 hours) between radiological marking and surgery, the dye spreads quickly into the surrounding area, so that large areas are stained, and exact detection is then no longer possible.

The sterile activated carbon that is suspended in physiological common salt solution, however, remains, for the most part, at the injection site until the operating surgeon removes it. This considerably facilitates scheduling between radiological marking and, e.g., a routine operation. In addition, an exact histopathologic correlation is generally possible without difficulty with good marking. Drawbacks of activated carbon include, however, the fact that the latter cannot be excreted from the body and can lead to reactive changes in the tissue or in draining lymph nodes. In addition, fine-particulate activated carbon also tends to lump up -- possibly due to electrostatic effects. This is a considerable drawback since needles of up to 18 gauge frequently clog and thus remarking with a new needle must be performed.

The object of this invention is therefore to provide a marking agent that overcomes the drawbacks of the prior art, i.e., to find a marking agent that

1. is well-tolerated,
2. exhibits immobility in the tissue over a sufficiently long period,
3. can be detected both visually and radiologically in the tissue in question, and that

4. is readily injectable through long and thin cannulae.

The object is achieved by the invention.

It has been found that black or colored contrast media are suitable for magnetic resonance tomography or diagnostic radiology, as well as gas-/dye- or gas-containing microparticles, such as are suitable for ultrasonic diagnosis, which readily meet the above-mentioned requirements, surprisingly enough, and are therefore extremely well suited for visually marking bodily tissues for the purpose of subsequent detection of such tissues.

The invention thus pertains to the use of colored NMR or x-ray contrast media or of optionally dye-containing ultrasonic contrast media for the production of a diagnostic agent for visually marking bodily tissues.

Examples of such agents are mentioned below.

Suitable are, e.g., suspensions of magnetites, i.e., colloidal solutions or mixtures of FeO and Fe_2O_3 that are stabilized by a more or less hydrophilic coating with generally organic molecules. Because of their strong effect on the relaxation of protons or owing to their x-ray-absorbing action, the latter show not only an imaging effect in radiological processes, they are also readily visually detectable because of their dark brown to black colors, so that they are especially well suited for marking light tissues (e.g., fat, glandular tissue, muscle, connective tissue). Although magnetites almost always have diameters of far below 1 μm and are readily suspendable in water and thus transportable, they remain largely undiluted at the injection site for a surprisingly long time

(days to months) after injection into the tissue, and they are readily visible there to the eye. Magnetites are locally well-tolerated. Iron oxides are dissolved on a long-term basis, and iron is converted in natural iron metabolism.

In addition, marking with magnetites opens up the possibility of dyeing in histologic sections the iron that is contained in the magnetites with potassium rhodanide as "Berlin blue."

Magnetites that are suitable for medical use and processes for their production are described, e.g., in US 4,731,239, US 4,770,183, US 4,827,945, US 4,767,611, EP 0 186 616, SE 83 070 60, SE 84 054 99, GB 84-08127, DE 35 79 899, WO 91/05807.

In addition to magnetites, metal porphyrins are also suitable for the above-indicated purpose. The latter can be readily detected, depending on the metal contained, either by radiography or, if these are paramagnetic metal ions, such as, e.g., Fe^{3+}, manganese^{3+}, gadolinium^{3+}, etc. in the complexed metals, with the aid of magnetic resonance tomography. In addition, porphyrins absorb light in the visible frequency range, so that they can also be readily detected visually. Especially suitable are intensively colored complexes, as they are described, e.g., in EP 0 336 879 or EP 0 355 041.

Further suitable are ultrasonic contrast media that also contain a dye in addition to the encapsulated echoing gas. Such contrast media are described in DE 43 30 958. As dyes, basically all physiologically compatible dyes, such as, e.g., hemoglobin, chlorophyll, etc., are suitable. Suitable also, however, are

those dyes which would quickly diffuse in unencapsulated form (such as, e.g., methylene blue). Encapsulation prevents diffusion, i.e., the dye remains at the injection site and can be released there only just before surgery, as described in DE 43 30 958, by irradiation of ultrasound of suitable frequency and wavelength.

Since ultrasonic devices are available in any operating room, the release of dye can even be done by surgeons in the operating room. Extensive dispersion of dye is largely avoided because of the brevity of the period between the release of dye and the visual redetection of the marked points even in the case of water-soluble dyes that diffuse quickly.

Other particles that can be detected by ultrasound, such as are described, e.g., in US 4,276,885, EP 0 327 490, EP 0 458 079 and EP 0 535 387, are also suitable for marking.

In addition, various fairly non-toxic dyes that can be detected in the body by imaging processes that are commonly used, such as, e.g., melanin or various stable radicals that influence the relaxation times of tissues and therefore result in an imaging effect in nuclear spin tomography, are suitable. Examples of such radicals include [G. Sosnovsky, A Critical Evaluation of the Present Status of Toxicity of Aminoxyl Radicals, J. Pharmaceut. Sci. 81 (1992) 496-499].

The previously mentioned agents are extremely well suited for marking tissues, especially in regions of the body that are not accessible to direct visual inspection. Surprisingly enough, they are also stable in place over a prolonged period, i.e., they

do not diffuse during the interval of time between marking and surgery or diffuse negligibly slowly. The above-mentioned agents are well-tolerated and visually detectable, surprisingly enough, even at very low concentrations while still being usable in imaging radiological processes. As a result, with a dose to be administered in an injection it is possible both to prepare a control picture with an imaging process [such as, e.g., radiotechnology including conventional x-ray pictures, such as, e.g., mammography or computer tomography, magnetic resonance tomography (MRT) as well as ultrasound methods], and to produce a marking that is readily discernible to surgeons.

The above-mentioned agents are thus of special value in the marking of small tissue areas that lie one or more centimeters below the body surface area, especially if the latter -- owing to their position in soft tissue -- can easily slide in the case of movement (e.g., breast tissue, testicular tissue).

They are also especially suitable for marking the spot of the tissue sample that is removed before the surgical intervention (under the control of the mentioned imaging processes) using a biopsy needle. Without such a marking, this spot could not be found again in a surgical intervention that may be necessary later after the needle is pulled out.

In addition, especially in the case of small lesions, the difficulty of placing the biopsy needle exactly often exists, so that not suspect tissue -- as presumed -- but rather surrounding healthy tissue is removed, which necessarily results in a false pathological finding. If the above-mentioned agent is injected,

however, immediately after the removal of the sample via the same cannula, it can be readily discerned in the corresponding imaging process whether the sample was actually removed at the intended spot.

According to the invention, the above-mentioned agents are generally introduced via a fairly long cannula as a solution, suspension or emulsion, while being observed visually in the above-mentioned imaging processes in the corresponding tissue region. If the imaging process used is nuclear spin tomography, cannulas that consist of non-magnetic material are to be used, of course, because of the strong magnetic fields. Owing to the imaging properties of the administered agent, a control picture can be produced immediately after administration which will show whether the marking was actually placed at the desired spot. Also, if the lesion was missed, the operating surgeon can easily find the tumor region again based on the color marking since the relative position of the tumor region in the color marking can be easily ascertained from the control picture.

As already mentioned, the above-mentioned agents are generally readily visible to the naked eye. In the case of ultrasonic contrast media based on gaseous microparticles, it is advisable, however (because of the insignificant marking caused by the particles), to redetect particles with an ultrasonic scanner instead of visual inspection. Such devices are available in any operating room (unlike a nuclear spin tomograph or an x-ray device).

In the case of some marking agents, such as, e.g., porphyrins, it is advisable to enhance the chromophoric effect by stimulation with infrared or ultraviolet light. Also, the additives required for this are readily available in any operating room.

All preparations that are provided for injection into tissue must be absolutely sterile. They may optionally contain the pharmaceutical adjuvants that are commonly used for isotonizing (e.g., glucose, NaCl), buffering or stabilizing the solution or emulsion or suspension.

The dose administered depends primarily on the size of the tissue area to be marked. It may be very small at 50 μl. Injection volumes of 0.1 to 2 ml are preferred. In the case of sizeable or multifocal processes, it can be necessary to inject up to 5 or more milliliters of marking agent. In the case of low volumes, higher concentrations of the individual components are preferred; in the case of higher volumes, smaller concentrations are more likely to be used in order to avoid, among other things, artifacts in the imaging diagnosis.

The concentrations of the agents administered vary depending on the imaging processes selected in each case.

Thus, opacifying substances for magnetic resonance tomography are used at concentrations of 0.1-100 μmol/ml, preferably 1-20 μmol/ml, x-ray-opacifying agents are used in the range of 3-100 mg of opacifying elements (e.g., iodine, iron, etc.)/ml and ultrasonic-opacifying preparations in the range of 0.01 to 50 μl of gas/ml, preferably 0.1 to 5 μl of gas/ml.

Magnetites are also used as aqueous solutions with about 0.1 µmol of iron/ml to 500 µmol of iron/ml. Strongly colored solutions with about 20-500 µmol of iron/ml are preferred.

Metalloporphyrins are used in the same concentration range (0.1 µmol/ml - 500 µmol/ml), whereby the preferred range is approximately 20-200 µmol/ml.

Other paramagnetic or metal ion-containing or iodine-containing dyes, such as, e.g., Bengal pink, erythrosin, tetrachlorotetraiodine fluorescein, are preferably used at somewhat higher concentrations (50-500 µmol/ml).

The above-indicated concentrations are to be considered as guideline values; individual cases may exceed or fall below these guideline values. They are sufficient to produce a clear imaging effect and at the same time a visually discernible marking of the tissue in the respective radiological process.

The following examples are used to explain the object of the invention, without intending that they be limited to this object.

Example 1 Marking of a Biopsy Area with Magnetites

A suspect tissue region is identified in MRT. To study tumorous tissue, a needle biopsy is performed, in which cells are obtained from the corresponding area. To control the exact position of the needle as well as to facilitate finding the appropriate tissue region again in the case of a subsequent operation, 0.5 ml of a 50 mmol dextran-magnetite solution (SH U 555, Schering AG, Berlin) is injected. After the solution is injected, the appropriate area in the MRT represents a signal alarm. In the laying-open during surgery, the biopsy area can be discerned from the brownish discoloration. In the same way, the pathologist can get his bearings in the surgery and apply the necessary sections in the proper plane.

Example 2 Marking of a Biopsy Area with Porphyrins

The procedure is as described in Example 1. As a marking solution, however, 2 μmol of manganese (as manganese(III) {tetrakis-[3-(carboxylatomethoxy)-phenyl]-porphyrin}-acetate complex) is injected in a milliliter of physiological common salt solution. In the laying-open during surgery, the biopsy area can be discerned from the yellow-greenish discoloration. The color effect can be additionally enhanced by short-term stimulation with UV light.

Example 3 Marking of a Breast Cancer with Magnetites

During mammography, microcalcifications are found at a volume of less than 0.5 cm^3. The region is stereotactically

biopsied. At the same time, 0.2 ml of 500 mmol magnetite solution is injected. The biopsy reveals a breast cancer. The identification of the lesion during surgery is facilitated by the clear black-brown discoloration.

Example 4 Marking of Muscle Tissue with Magnetites

The dextran-magnetite solution (SH U 555, Schering AG) is injected at a concentration of A) 500 mmol of Fe/liter, B) 250 mmol of Fe/liter, C) 125 mmol of Fe/liter and a dose of 1 ml in muscle tissue and examined by computer tomography. The magnetite depot is clearly discernible in all cases and strictly limited in shape. After the corresponding tissue region is laid open, the magnetite is optically readily discernible from the brownish discoloration.

Example 5

Methylene blue-containing microparticles that consist of poly(D,L-lactic acid-glycolic acid) are irradiated in vitro in a tissue sample with ultrasound (sonic pressure > 50 dB, frequency 2.5 Mhz), and in this process methylene blue is released in the tissue.

The methylene blue-containing particles can be produced as disclosed in DE 43 30 958 (Example 8), by 4 g of poly(D,L-lactic acid-glycolic acid) (50:50) (Resomer RG 503, Boehringer Ingelheim), dissolved in 50 ml of CH_2Cl_2, being emulsified with 20 mg of methylene blue, dissolved in 4 ml of aqueous 4% gelatin solution, while being stirred with a fast stirrer. Then, another

200 ml of a 4% autoclaved gelatin solution is added. The emulsion is stirred for 8 hours at room temperature. The particles that are produced are filtered by a 5 μm filter, separated by centrifuging, resuspended in 50 ml of 4% autoclaved gelatin solution, frozen at -78°C, and freeze-dried. After resuspension, the gaseous microparticles are separated by centrifuging (at 1000 rpm, 30 min). The particles thus obtained are taken up in 20 ml of water for injection purposes and can be used directly for marking bodily tissue.

Example 6

0.05 ml of a suspension of gas-filled hollow bodies with a shell made of biodegradable poly-2-cyanoacrylic acid butyl ester (WO93/25242; Example 1) and an average particle size of about 2 μm is injected into the musculature of the upper leg of a dog. 3.5 hours later, a strictly limited depot of contrast medium, which can be readily located in all planes, is found in the ultrasonic study with a 3.5 MHz transducer.

Claims

1. Use of colored NMR or x-ray contrast media or of dye-containing ultrasonic contrast media for the production of a diagnostic agent for visually marking bodily tissues.

2. Use of ultrasonic contrast media for the production of a diagnostic agent for marking bodily tissues.

3. Use of an agent according to claim 1 that contains as a colored NMR contrast medium at least one metal porphyrin, magnetic iron oxide particles, nitroxide or melanin.

4. Use of an agent according to claim 1 that contains as a colored x-ray contrast medium Bengal pink, erythrosin or tetrachlorotetraiodine fluorescein.

5. Use of an agent according to claim 1 that contains as a dye-containing ultrasonic contrast medium microparticles that consist of a shell made of a biodegradable polymer and a gaseous and dye-containing nucleus.

6. Use of an agent according to claim 2 that contains as an ultrasonic contrast medium microparticles that consist of a polylactide-glycolide or polycyanacrylate shell and a gaseous nucleus.

7. Agents for visually marking bodily tissues that contain at least one colored, radiologically detectable substance.

8. Agents for marking bodily tissues according to claim 7 that contain magnetites as a colored, radiologically detectable substance.

9. Agents for marking bodily tissues according to claim 7 that contain metal complexes of porphyrins as a colored, radiologically detectable substance.

10. Agents for marking bodily tissues according to claim 7 that contain gas-containing microparticles, which optionally additionally contain a dye, as a colored, radiologically detectable substance.